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13. ABSTRACT *(Maximum 200 Words)*

We are testing the hypothesis that human mammary tumor virus (HMTV), a human endogenous retrovirus (HERV) closely related to mouse mammary tumor virus (MMTV), is etiologically involved in a subset of human breast cancers. We continue to collect blood and tissue from breast cancer patients, appropriate control subjects and from archival resources. We are determining the incidence of HMTV in these populations using polymerase chain reaction (PCR), and comparing the sequences of HMTV from different individuals to determine the extent of genetic variability. We have now constructed a genomic DNA library from a breast tumor positive for HMTV proviruses. Clones representing the entire HMTV genome, including flanking regions containing potential oncogenes, will be isolated and sequenced. Studies to express HMTV proteins and characterize the immunological reactions against these proteins in breast cancer patients and controls are in progress. The proposed studies will provide evidence indicative of whether or not HMTV is involved in breast cancer. HMTV may provide a target for vaccine development and breast cancer therapy.

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Table of Contents

Cover 1

SF 298..... 2

Table of Contents..... 3

Introduction..... 4

Body 5

Key Research Accomplishments..... 6

Reportable Outcomes..... 7

Conclusions..... 8

References..... 8

Appendices..... 9-13

INTRODUCTION:

Sequences with very high similarity (>90%) to mouse mammary tumor virus (MMTV) have been amplified by polymerase chain reaction (PCR) from human breast cancer (BC) tissue (Wang *et al., Cancer Research* **55:** 5173-5179, 1995). The authors of this study presented evidence that the genomic DNA of a subset (~38%) of human breast carcinomas, but not normal tissues, contains sequences that are very highly similar to the MMTV envelope gene (*env*). They suggested the existence of a human mammary tumor virus (HMTV) which is spread by the exogenous route of infection (horizontal transmission). We have PCR amplified sequences highly similar (>95%) to the MMTV *env* gene from human genomic DNA samples, including subsets of both BC tissue and nonBC tissues or blood. A ribonuclease protection assay was used to confirm this result using a non-PCR based technique and to determine that the majority of the PCR positive BC tissues, but none of the PCR negative BC tissues, expressed this sequence at the mRNA level. In addition to mice and humans, we amplified sequences from nonBC genomic DNA of a subset of cats and rhesus macaques distinct from, but highly related to, the MMTV *env*. We also amplified from cat DNA a sequence approximately 90% similar to the MMTV group antigen gene (*gag*). Our results differ from those of Wang and coworkers who, with few exceptions, were able to detect MMTV-like sequences only in breast tumors. Our results indicate that vertebrate species other than mice, including some humans, contain an endogenous retrovirus closely related to MMTV. Our overall goal is to determine whether or not HMTV is involved in a subset of human breast cancers. The first specific aim of this proposal is to recruit and clinically characterize cohorts of breast cancer and appropriate control patients. Various tissues will be obtained from subjects in these cohorts and from archival resources. BC tissues will be staged and classified by standard histological techniques. In studies proposed in the second specific aim, we will identify and sequence HMTV nucleic acids in breast cancer tissue, control tissue, and blood of patients from our cohorts. We will also determine the incidence of HMTV in these various control populations, and compare the sequences of several HMTV genes from different individuals to determine the extent of genetic variability. The third specific aim is to construct DNA or cDNA libraries from tissues positive for HMTV proviruses. These libraries will be screened to identify clones representing the entire HMTV genome. The clones will be sequenced to provide further evidence of the relationship of HMTV to other retroviruses. In studies under specific aim four, we propose to express HMTV proteins in an insect cell system which allows stable expression of recombinant proteins, and to characterize the immunological reactions of breast cancer patients and controls against HMTV proteins. The proposed studies will establish whether or not HMTV is involved in breast cancer. If a definitive link is established, HMTV will provide a target for vaccine development and breast cancer therapy.

BODY:

Task 1. Recruit and clinically characterize cohorts of breast cancer and appropriate control patients, Months 1-6.

a. We have achieved this goal. We have obtained blood and various tissues will be obtained from about 200 breast cancer and control subjects. BC tissues are being staged and classified by standard histological techniques.

Task 2. Identify and sequence of HMTV nucleic acids in breast cancer tissue, control tissue, and blood of patients from our cohorts, Months 3-15.

a. The incidence of HMTV in various breast cancer and control populations is being determined. HMTV env and LTR sequences have been detected in about 85% of BC tissues and in about 10-15% of healthy controls. 40 BC tissues have been examined and about 100 controls have been examined. This task is ongoing.
b. Sequences of HMTV from different individuals are being compared to determine the extent of genetic variability. About 30 different HMTV sequences have been obtained.
c. The level of expression of HMTV gene sequences, relative to that of housekeeping genes, by ribonuclease protection assay will be determined. This task is ongoing.
d. The initial publication based on PCR based molecular epidemiology is being written and submitted.

Task 3. Construct and screen genomic DNA or cDNA libraries from tissues positive for HMTV proviruses, Months 15-24.

a. A DNA library has been constructed in Lambda ZAPII from an HMTV positive tumor.
b. The DNA library is being screened by colony hybridization and PCR to identify clones representing the entire HMTV genome. This task is ongoing.
c. HMTV specific clones will be sequenced to provide further evidence of the relationship of HMTV to other retroviruses and other information. This task is ongoing.
d. Publications based on the complete HMTV sequence will be written and submitted when this task is completed.

Task 4. Characterize immune reactions to HMTV proteins, Months 25-36.

a. HMTV proteins will be expressed in an insect cell system that allows stable expression of recombinant proteins. This task has been initiated.
b. Immunoassays using HMTV recombinant proteins will be developed and validated.
c. Immunological reactions of patients with breast cancer and various control subjects will be characterized.
d. Publications based on results from HMTV serological testing will be written and submitted when this task is completed.

KEY RESEARCH ACCOMPLISHMENTS:

- We have obtained blood and various tissues will be obtained from about 200 breast cancer and control subjects. BC tissues are being staged and classified by standard histological techniques.

- The incidence of HMTV in various breast cancer and control populations is being determined. HMTV env and LTR sequences have been detected in about 85% of BC tissues and in about 10-15% of healthy controls. 40 BC tissues have been examined and about 100 controls have been examined. This task is ongoing.

- Sequences of HMTV from different individuals are being compared to determine the extent of genetic variability. About 30 different HMTV sequences have been obtained

- A DNA library has been constructed in Lambda ZAPII from an HMTV positive tumor.

- The DNA library is being screened by colony hybridization and PCR to identify clones representing the entire HMTV genome.

- We have initiated studies to express HMTV proteins in an insect cell system that allows stable expression of recombinant proteins. .

REPORTABLE OUTCOMES:

Manuscripts:

Garry, R.F. Human Mammary Tumor Virus. In: Where We Stand with Breast Cancer Research (N.J. Agnantis and D.D. Tsiftsis, Eds.) Synedron Press (Athens, Greece), 154-155, 1999.

Identification of mouse mammary tumor virus related sequences in the human genome. Soble, S.S., Pei, B., Nangle, S., Costin, J. , Crawford, B.E., Haislip, A.M. and Garry, R.F. in preparation.

Abstracts:

Pei, B. and Garry, R.F. Identification of mouse mammary tumor virus related sequences in the human genome. Presented at Molecular and Cellular Biology annual Retreat October 14, 2000.

Costin, J.M. S. Szabo, S., Haislip, A.M., McCracken, J.P., Nangle, S.E., Traina-Dorge, V. Crawford, B.E. II, Hill, S.M., and Garry, R.F. Human sequences related to mouse mammary tumor virus. Presented at Tulane Research Day November 2000.

Soble, S.S., Haislip, A.M., Hill, S. M., and Garry, R.F. 1999. Human sequences related to mouse mammary tumor virus. Presented at the International Congress of Virology

Presentations:

Viruses and Human Cancer: New Associations. Fred Hutchinson Cancer Center. Seattle, Washington. Invited Symposium Speaker. (0ctober 18th)

Viral Cause of Human Breast Cancer. North Shore University Hospital, Manhasset, New York. Invited Symposium Speaker. (March 10th)

Patents and licenses applied for and/or issued: none

Degrees obtained that are supported by this award: none

Development of cell lines, tissue or serum repositories:

We have obtained blood and various tissues from about 200 BC and control subjects

Infomatics: not applicable.

Funding applied for based on work supported by this award: none

Employment or research opportunities applied for and/or received: none

CONCLUSIONS: We continue to make timely progress on each of the specific tasks outlined in the study. Because of this progress, there are no recommend changes on future work to better address the problem. There remains every indication that current and proposed studies will establish whether or not HMTV is involved in breast cancer. If a definitive link is established, HMTV will provide a target for vaccine development and breast cancer therapy.

REFERENCES: none

Presented at the International Congress of Virology
August 1999

HUMAN SEQUENCES RELATED TO MOUSE MAMMARY TUMOR VIRUS. Sara S. Soble,[1] Allyson M. Haislip,[2] Steven M. Hill,[1.3] and Robert F. Garry.*[,1.2] Graduate Program in Molecular and Cellular Biology,[1] Departments of Microbiology and Immunology,[2] and Anatomy,[3] Tulane University School of Medicine, New Orleans, LA, USA

Sequences with very high similarity (>90%) to mouse mammary tumor virus (MMTV) have been amplified by polymerase chain reaction (PCR) from human breast cancer (BC) tissue (Wang *et al., Cancer Research* **55:** 5173-5179, 1995). The authors of this study presented evidence that the genomic DNA of a subset (~38%) of human breast carcinomas, but not normal tissues, contains sequences that are very highly similar to the MMTV envelope gene (*env*). They suggested the existence of a human mammary tumor virus which is spread by the exogenous route of infection (horizontal transmission). We have PCR amplified sequences highly similar (>95%) to the MMTV *env* gene from human genomic DNA samples, including a subset (~50%) of BC tissues. A ribonuclease protection assay was used to confirm this result using a non-PCR based technique and to determine that the majority of the PCR positive BC tissues, but none of the PCR negative BC tissues, expressed this sequence at the mRNA level. In addition to mice and humans, we amplified sequences from non BC genomic DNA of a subset of cats and rheusus macaques distinct from, but highly related to, MMTV *env*. We also amplified from cat DNA a sequence approximately 90% similar to the MMTV group antigen gene (*gag*). Our results differ from those of Wang and coworkers who, with few exceptions, were able to detect MMTV-like sequences only in breast tumors. Our results indicate that vertebrate species other than mice, including some humans, contain an endogenous retrovirus closely related to MMTV.

Presented at Molecular and Cellular Biology annual Retreat October 14, 2000

Identification of mouse mammary tumor virus related sequences in the human genome

Baikang Pei and Robert F. Garry. Department of Microbiology and Immunology, Tulane Medical School.

Abstract

Mouse mammary tumor virus (MMTV), a member of the retrovirus family , can cause breast cancer in several kinds of laboratory mice. Sequences with very high similarity (>90%) to MMTV have been amplified by polymerase chain reaction (PCR) from human breast cancer (BC) tissue, but the authors of this study did not find sequences highly similar to MMTV in normal tissue. They suggested that the existence of these sequences in BC tissue is due to exogenous infection. Thus, no endogenous sequence with very high homology to MMTV has been detected. It was of interest to see if there is such a homogeneous sequence, human mammary tumor virus (HMTV), in the human genome, and if that sequence has some relationship with mammary cancer. In our lab, we have also found evidence for a human retrovirus that is very similar to MMTV. However, we found HMTV in both breast cancer tissue and tissue from other organs and in the blood. We also identified HMTV in tissues and blood from people without breast cancer. The technologies we used are mainly PCR and Southern blot. These results indicated that humans contain an endogenous retrovirus closely related to MMTV. These endogenous retroviruses are not typically spread by the infectious routes, but rather are inherited. After finding the endogenous retroviruses in human genome, future studies will involve sequencing the entire HMTV genome and characterizing immune reactions to HMTV proteins. This work will establish whether or not HMTV is involved in breast cancer.

PRESENTED AT TULANE RESEARCH DAY NOVEMBER 2000

HUMAN SEQUENCES RELATED TO MOUSE MAMMARY TUMOR VIRUS.

J.M. Costin,[1] S. Szabo,[2,3] A. M. Haislip,[1] J. P. McCracken,[1] S.E. Nangle,[4] V. Traina-Dorge,[2,5] B.E. Crawford, II,[3] S.M. Hill,[2,6] and R.F. Garry[1.2] Microbiology and Immunology,[1] Molecular and Cellular Biology,[2] Pathology and Laboratory Medicine,[3] Structural and Cellular Biology,[6] Tulane University School of Medicine and Public Health and Tropical Medicine[4], New Orleans, LA, and the Tulane Regional Primate Research Center, Covington, LA.[5]

Objective: The existence of a human retrovirus closely related to mammary tumor virus which is spread by the exogenous route of infection has been suggested by Wang and coworkers (*Cancer Research* **55:** 5173-5179, 1995). Studies were undertaken to confirm and extend these findings.

Experimental plan and results: We have PCR amplified sequences highly similar (>95%) to the MMTV *env* gene from human genomic DNA samples, including a subset of BC tissues. In addition to mice and humans, we amplified sequences from non BC genomic DNA of a subset of cats and rheusus macaques distinct from, but highly related to, MMTV *env.* We also amplified from cat DNA a sequence approximately 90% similar to the MMTV group antigen gene (*gag*).

Conclusion: Our results differ from those of Wang and coworkers who, with few exceptions, were able to detect MMTV-like sequences only in breast tumors. Our results indicate that vertebrate species other than mice, including some humans, contain an endogenous retrovirus closely related to MMTV.

2.40. HUMAN MAMMARY TUMOR VIRUS

Robert F. Garry

Mouse mammary tumor virus (MMTV) was discovered by Bittner in the 1930's as a outgrowth of studies of hereditary cancer in mice. Generally, MMTV is transmitted in the germline as an endogenous provirus (es). but MMTV may also be transmitted exogenously via the milk of infected dams. 30 or more unique proviral integration sites for endogenous MMTV have been identified in the genomes of various mouse strains, though it is important to note that some strains contain no endogenous MMTV proviruses. This suggests that MMTV may have the germline of Mus musculus after speciation. Some endogenous MMTV can be activated by hormones to form infectious virions capable of inducing mammary carcinomas via insertional mutagenesis.

The discovery of MMTV prompted many investigators to investigate a retroviral etiology for breast cancer (BC) in humans. Data collected over the past six decades has suggested the possible existence of a human homologue of MMTV. However, despite numerous electron microscopic, immunological, biochemical and molecular studies on human breast carcinoma tissue, milk, patients' sera, and breast carcinoma cell lines suggesting the existence of a human homologue of MMTV, proof that such an agent exists has remained elusive. Most authors have dismissed the importance of prior studies purporting to show evidence of a human mammary tumor virus because of he presence in the human genome of the human endogenous retrovirus-K family (HERV-K), whose members shows about 60% sequence similarity to MMTV.

Recently sequences with very high similarity to those of MMTV been isolated from human BC tissue (Wang et al, 1995). Sequences 95-99% similar to the MMTV envelope gene (env) were detected by PCR in 121 (38.5%) of 314 unselected breast cancer tumor samples. It is permanent to note that the MMTV-like sequences were detected in only 2 (1.8%) of 107 breast specimens from reduction mammoplasties and in 0/80 samples from normal tissues or non-breast tumors. The MMTV-env like RNA was expressed (as determined by RT-PCR) in 66% of DNA PCR positive breast tumors (Wang et al, 1998). A complete 9.9 Kb provirus with 94% similarity to MMTV was detected in 2 breast tumors. FISH revealed integration at several sites in BC DNA, but not normal breast cells (Wang et al, 1999 ACR mtg. abstracts #2933, 2944). Thereby, Wang and co-workers suggested the existence of a human mammary tumor virus (HMTV) spread by the exogenous route of infection (horizontal transmission). These and other investigators' attempts to amplify env and other regions of the MMTV-related virus from genomic DNA or cDNA of subjects free of BC yielded sequences, including HERV-K10 and related retroviral elements, with only up to about 60% sequence similarity to MMTV. Thus, no endogeneous (germline or vertically-transmitted) sequence with very high homology to MMTV had been detected prior to the studies described herein.

We have PCR amplified sequences highly similar (>95%) to the MMTV env gene from human genomic DNA samples, including subsets of both BC tissue and non-BC tissues. We found HMTV sequences by PCR and a sensitive blotting technique not only in the substantial majority of breast tumors, but also in the blood of smaller subsets of systemic lupus erythematosus patients and healthy controls without BC. The sequences obtained from these human subjects were distinct from MMTV sequences used as controls in these PCR reactions indicating that these results using a non-PCR based technique to determine that the majority of the PCR positive BC tissues, but none of the PCR negative BC tissues, expressed this sequence at the mRNA level. We have also sequenced many of the products from our PCR reactions. Analysis of these sequences provides further strong evidence that PCR contamination is an unlikely explanation for our results. MMTV env-like sequences from different individuals derived in the same PCR run were distinct from each other. This result indicates that lack of a ubquitous PCR contaminant that would have produced a more consistent sequence that should have been identical (or nearly so except for recognizable Taq polymerase errors) in the various reaction tubes. Furthermore DNA of individual subjects

produced consistent MMTV env-like sequences from PCR run to PCR run. The variations within HMTV from a given patient may represent a low number of Tag errors, but are also suggestive of variations expected of a replicating retrovirus. In addition to mice and humans, we amplified sequences from nonBC genomic DNA of a subject of cats and of rhesus macaques distinct from, but highly related to, MMTV env. A variety of tissues from other species were negative in these PCR assays. We also amplified from cat DNA a sequence approximately 90% similar to the MMTV group antigen gene (gag).

HERVs have recently been implicated in several important human diseases including multiple sclerosis, various autoimmune diseases, congenital heart block, and testicular and other germ cell cancers (Garry et al., 1990: reviewed in Garry, 2000). The current results indicate that vertebrate species other than mice, including some humans, can contain an endogenous retrovirus closely related to MMTV. Although these results should e interpreted with cautious (for example, the resent suggestion that type I diabetes is linked to HERV expression could not be confirmed), the close sequence similarity with MMTV provides further stimulus to define the role, if any, of HMTV in human breast cancer.

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